NEUROSECRETORY CELL TYPES AND THEIR SECRETORY ACTIVITY IN THE CRAYFISH [1, 2]

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It is now well known that physiologically active substances are produced in neurosecretory cells located throughout the nervous systems of crustaceans (Bliss, 1951, 1952, 1953; Bliss, Durand and Welsh, 1954; Bliss and Welsh, 1952; Carlisle, 1953; Enami, 1951; Passano, 1951a, 1952, 1953). Furthermore, the neurosecretory cells are distributed as distinct groups (Bliss, Durand and Welsh, 1954; Enami, 1951), at least in the eyestalk and brain. Relatively little is known about the specific localization of the sources of the neurohormones affecting particular physiological processes; however, Passano (1951a, 1951b, 1952, 1953) has shown that the x-organ in crustaceans produces a substance that is capable of inhibiting molt.

Neurosecretory cells have been described for the x-organ (Bliss, 1952; Bliss, Durand and Welsh, 1954; Bliss and Welsh, 1952; Carlisle and Passano, 1953; Enami, 1951; Passano, 1953); but, with the exception of Enami's work on *Sesarma* (1951), there is little information concerning the different types of neurosecretory cells present in crustaceans. Furthermore, there is no cytological evidence available to indicate which of the different neurosecretory cell types are involved in the physiology of molt. It is apparent that work along these lines is needed, particularly in view of the fact that cytological differences in cell types often go hand in hand with differences in function.

The present paper will be concerned with a histological study of the neurosecretory system of the crayfish, *Orconectes virilis* (formerly *Cambarus virilis*) in relation to the molting cycle.

Materials and Methods

1. *Animals*

The animals used in this study were mature males, approximately five centimeters in carapace length, all collected from Hobb's Brook Reservoir, Lincoln, Mass., in the summer of 1954. Mature crayfish were collected on the dates shown in Table I. With the exception of May animals, which had been kept in the laboratory for three to four months and fed weekly on clam and fish, eyestalks and brains were removed and fixed on the same day the animals were collected.

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2. Dissections

All dissections were performed in crayfish perfusion fluid (van Harreveld, 1936). A pair of fine iridectomy scissors, jeweler's forceps and cuticle scissors were used in the dissections.

Eyestalk. The eyestalk was removed by cutting the pedunculus lobi optici with a pair of small cuticle scissors. Next, the chitinous exoskeleton was cut the full length of the eyestalk on each side. The eyestalk was then pinned ventral side down by means of size 0 insect pins in a Syracuse watch glass, half filled with paraffin and containing crayfish perfusion fluid. The remainder of the dissection was carried out with the aid of a binocular dissecting microscope.

After a cut was made across the dorsal half of the retina, the proximal end of the top half of the exoskeleton was lifted and the hypodermis was carefully scraped from the exoskeleton. Great care was taken in this step to prevent excess stretching of the nerve tissue.

The cut end of the pedunculus lobi optici was grasped with fine forceps, and the eyestalk contents were separated from the underlying exoskeleton. The whole content of the eyestalk was then placed in a vial containing fixative. With practice, this procedure could be accomplished within two to three minutes. Excellent fixation was obtained in all cases.

Brain. The head of the animal was removed by a cut just posterior to the brain and mouth. The exposed parts were immediately rinsed thoroughly with perfusion fluid to remove any stomach contents, pieces of hepatopancreas, or urine released after puncture of the bladders. Frequent changes of the perfusion fluid were made throughout the dissection. The rostrum of the animal was next inserted in a piece of modeling clay in such a manner that the open end of the head was facing up. In this way, the animal's head served as a miniature dissecting vessel. The remainder of the procedure was carried out with the aid of a dissecting microscope.

After removal of the stomach, pieces of hepatopancreas, and green glands, the brain was rinsed thoroughly with perfusion fluid. All nerves leading from the brain and the connective tissue sheath surrounding the brain were cut away, and the brain was placed in a vial containing fixative. The brain was lifted by means of the circumoesophageal connectives. This procedure required about three to four minutes.

3. Histological procedure

The fixatives employed in this study were Helly's fluid (fixing time, eight hours) and Bouin's plus one per cent calcium chloride (fixing time, twenty-four hours). Tissues were dehydrated in alcohol, cleared in cedar oil and embedded in Tissuemat (melting point 56–58° C.). Sections were cut at 6 μ and stained with aldehyde fuchsin (Gomori, 1950) according to the schedule of Halmi (1952), but with modifications by Dawson (1953). This procedure involved a permanganate oxidation prior to staining and will be referred to in the text as PAF. Sections were also stained with chrome-alum-hematoxylin-phloxin (Gomori, 1941) as adapted by Bargmann (1949). This technique is referred to in the text as CHP.

4. *Cell counts*

A study of cell types revealed that secretory material was present as small granules or droplets within the cells. The secretory activity of a group of cells could be judged, therefore, by counts of cells which appeared histologically to be in a given stage of the secretory cycle.

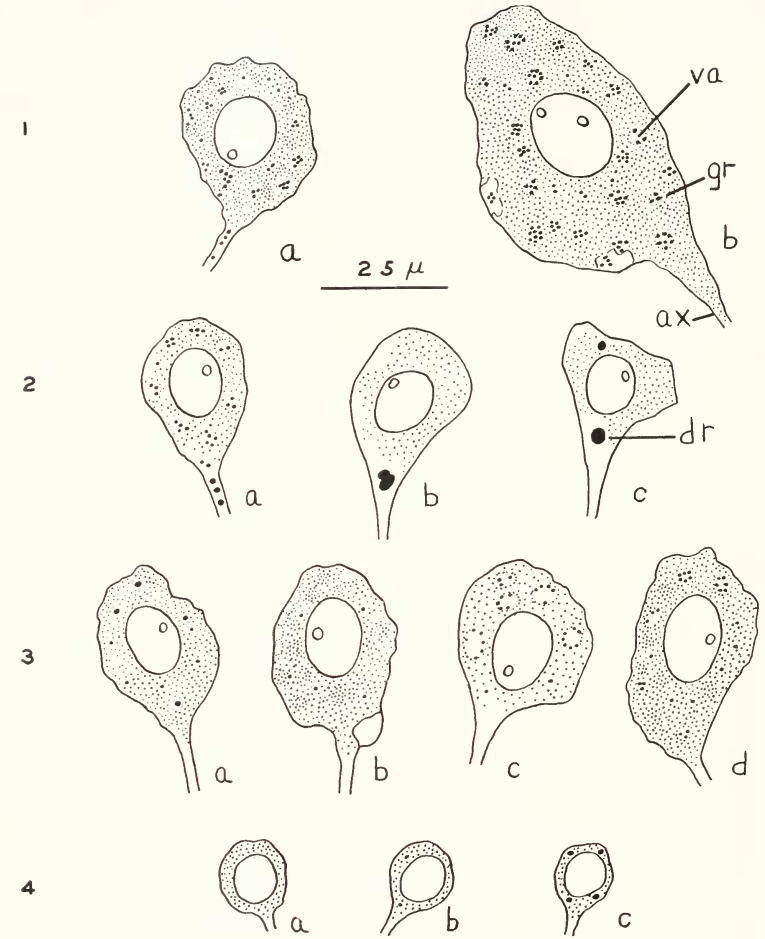


FIGURE 1. Drawings of neurosecretory cell types in the eyestalk and brain of the crayfish. Numbers along the left column indicate cell types. Letters indicate cells in successive stages of the secretory cycle. ax, axon; dr, droplet; gr, granule; va, vacuole.

Type 1 cells of the x-organ, in a stage of the secretory cycle similar to that shown in Figure 2, were counted. These cells are large enough so that they can be recognized from section to section and were counted only when the nucleus was included in the section. In this way no cell could be counted twice.

Type 2 cells in the x-organ were also counted. In this case, cells which contained both a nucleus and a secretory droplet (Fig. 1, Cells 2b, c) in the same section were counted. The nuclei of these cells are small enough so that a section near the center of the nucleus would be present only once per cell. This method of counting resulted in minimum counts of the cells in that particular stage of the secretory cycle. Type 2 neurosecretory cells as shown in Figure 1, Cell 2a, were not counted. The marked uniformity of cell counts during all months except May and June indicates that consistent results can be obtained in this manner.

This method of counting could not be applied to the other neurosecretory cell groups because the secretory material in those groups is freely distributed throughout the cytoplasm in the form of fine granules (see below).

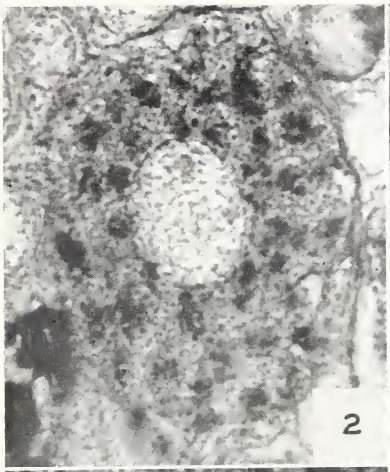
Results

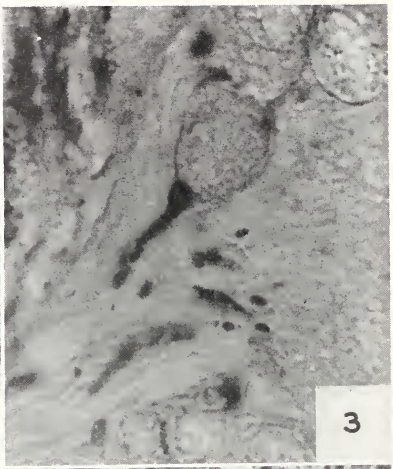
Studies of serial sections of eyestalks and brains, stained with CHP and PAF, have revealed the presence of large groups of cells (see also Bliss, Durand and Welsh, 1954) that are histologically different from the hundreds of ordinary ganglion cells present throughout the eyestalk and brain. These cells are always larger than the ordinary ganglion cells. Most of them possess large nuclei, have abundant cytoplasm, and are characterized by the presence in the perikaryon and axon of droplets of a material which stains conspicuously with aldehyde fuchsin and chrome-hematoxylin. Not all the cells have the same appearance as to the quantity and size of these droplets. These characteristics lead to the conclusion that the cells are neurosecretory cells as defined by E. Scharrer and B. Scharrer (1945) and described in a great variety of animals by numerous authors (see especially Scharrer and Scharrer, 1954; Gabe, 1954).

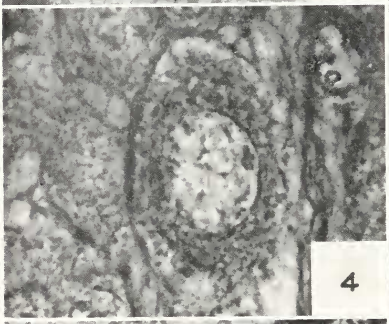
There appear to be four neurosecretory cell types (Fig. 1) found in the eyestalk and brain of the crayfish, *O. virilis*. Size, general shape of the cell body, presence or absence of vacuoles in the cytoplasm and the appearance of the secretory product were used as the main criteria in separating the cell types. Since large numbers of the cells were found to form more or less distinct subdivisions of larger units in the case of two cell types, and possessed a fairly uniform set of the characteristics listed above, it is believed that the cells are truly of different types and have not been confused with various stages of the secretory cycle present in a given cell type. The only cells that others might possibly find difficult to recognize are those similar to Type 1 (a) and Type 3 (d) (Fig. 1).

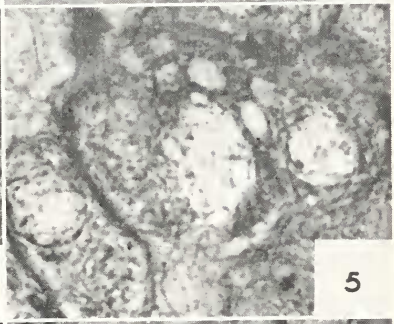
Cell types

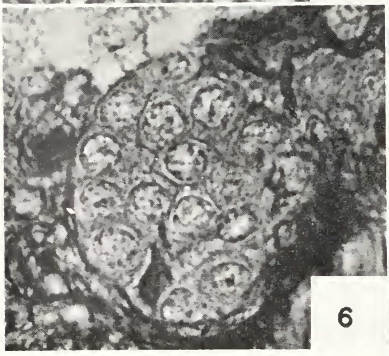
Cell Type 1. The distribution of this cell type is somewhat limited; it is most numerous in the x-organ and lies as a distinct subgroup in the most distal portion of the x-organ. The cell bodies are large, 40–60 μ in length, possess much cytoplasm and contain a large nucleus, 15 μ in diameter. In the material used in this study, Type 1 cell bodies have extremely irregular outlines which are very likely caused by shrinkage during fixation (Fig. 1). The nucleus may often contain two and some-

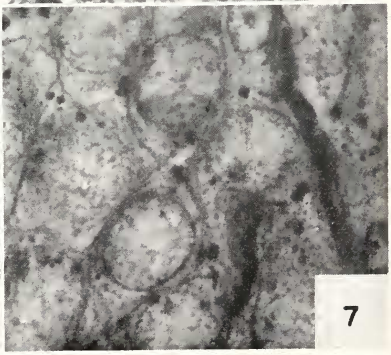












FIGURES 2–7.

times three nucleoli. Moreover, nucleoli are usually peripheral, lying against the nuclear membrane. Further, not all of the cells show the presence of large amounts of secretory material at any one time. This, however, is to be expected, for apparently some cells are at the peak of their secretory processes while others are in a quiescent state. The secretory product consists of a great number of aggregations of small (0.5–1.0 μ) granules that stain with aldehyde fuchsin (Figs. 1 and 2). It frequently appears as though the aggregations are located on the surface of small clear spaces in the cytoplasm. In cells that do not contain large amounts of secretory material, aggregations may not be present. In these cases secretory material is scattered in the cytoplasm as fine granules about the size of those that make up the aggregations. The cytoplasm is generally flaky in appearance and, in cells containing many granules, may sometimes be stained a red-purple by the PAF technique. However, many of the cells do not show this cytoplasmic staining; this is probably because the cells are in different stages of the secretory cycle.

In some sections, secretory material may also be seen at a point where the axon leaves the cell body and along the axon for a short distance. When it is found along the axon, the secretory material appears as a number of small granules strung out along the axon. Farther from the cell bodies, though, it appears to consist of a more finely divided suspension somewhat dispersed in the axons.

Cell Type 2. This cell type is a smaller cell which is also restricted in its distribution. These cells are arranged in the proximal part of the x-organ as a cluster of grapes as described by Hanström (1931). The cell body measures about 30 μ in length and is slightly narrower, 20–25 μ, than it is long (Fig. 1). It possesses a large nucleus, but none has been observed to contain more than one nucleolus. The nucleolus here is also near the nuclear membrane. The cytoplasm is somewhat vacuolated, although the vacuoles appear to be a result of fixation; they do not possess any definite shape. In February, these cells possess small vacuoles with granules of secretory material located peripherally. Some cells in February have granules contained within vacuoles. At other times of the year the material is present as quite large, 4 μ, distinct droplets (Figs. 1 and 9) as contrasted with the granules present in Type 1 cells. The droplets, usually one or two per cell, are almost always round and are usually located in the axon hillock or in the axon. Sometimes many drops may be seen along the bundles of axons as they leave the cell group (Fig. 3).

FIGURE 2. Type 1 neurosecretory cell in advanced stage of the secretory cycle. Note the aggregations of granules. Cells with this appearance were counted as indicating the secretory activity of this cell type. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin, 1300 ×.

FIGURE 3. Type 2 neurosecretory cell containing many droplets of secretory material in its axon hillock. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 1300 ×.

FIGURE 4. Type 3 neurosecretory cell showing peripheral arrangement of vacuoles (top of photograph). Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 1300 ×.

FIGURE 5. Type 3 neurosecretory cell. Note centrally located vacuole with granules of secretory material located on the surface. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 1400 ×.

FIGURE 6. Group of Type 4 neurosecretory cells in the eyestalk. Note scanty cytoplasm and scarcity of secretory material. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 700 ×.

FIGURE 7. Group of Type 4 neurosecretory cells in the brain. Note large content of secretory material. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 1200 ×.

Cell Type 3. This cell type is distributed freely throughout the neurosecretory cell groups in the eyestalk and brain with the exception of the x-organ. These cells are, on the average, slightly larger than the Type 2 cells (Figs. 1, 4 and 5). They are generally tear-drop shaped, although not as distinctly so as the Type 2

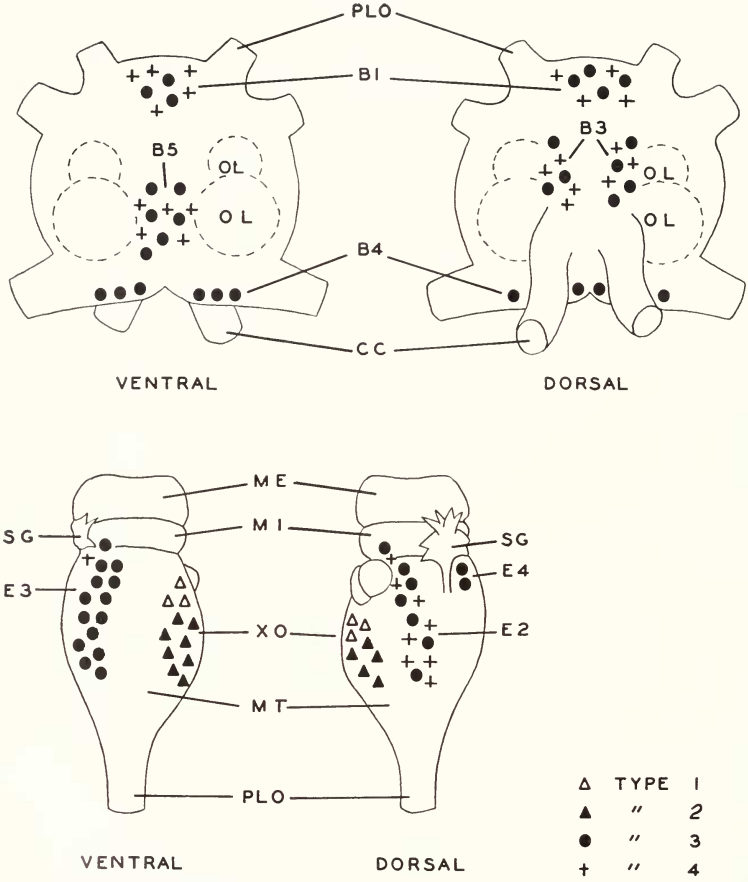


FIGURE 8. Diagrammatic representation of the distribution of neurosecretory cell types in the brain and eyestalk of the crayfish. Compare with Enami's Figure 11 (1951). B1 through B5 designate groups of neurosecretory cells in the brain. E1 through E4 designate groups of neurosecretory cells in the eyestalk. CC, circumoesophageal connectives; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; OL, olfactory lobes; PLO, pedunculus lobi optici; SG, sinus gland; XO, x-organ.

cells. Two characteristics distinguish these cells from the Type 2 cells. The first characteristic is the nature of the vacuoles. The vacuoles are rather large, up to 7 μ, and most of the time may be seen around the periphery of the cell (Figs. 1 and 4) although sometimes they may be located more centrally in the cytoplasm (Figs. 1 and 5). The vacuoles are sharply delimited from the cytoplasm. In this way they are markedly different from those usually found in Type 2 cells. The second characteristic is the appearance of the secretory product. Thus, in the Type 3 cells, the secretory product consists of fine granules which are never clumped in as large numbers as they are in the Type 1 cells (Fig. 2). Furthermore, secretory material is never present in the form of large droplets as it is in Type 2 cells. Granules are scattered, apparently at random, throughout the cytoplasm or they may be found on the surface of vacuoles or, sometimes, as a small drop in the center of one of the vacuoles (Fig. 1). Vacuolated cells of this type are sometimes found to contain no signs of secretion.

Cell Type 4. Cells of this type are located in all neurosecretory cell groups of the eyestalk and brain except the x-organ and group E3 (Fig. 8). The Type 4 cells are small, about 13 μ in diameter, possess a small nucleus, 10 μ in diameter, and, as is obvious from the measurements, very little cytoplasm (Figs. 1 and 6). They are classified as neurosecretory cells since preliminary studies show that, under certain conditions, some of the cells undergo changes in the amount of secretory material they contain (Fig. 7). Furthermore, they are similar to the gamma neurosecretory cells described by Enami (1951) and are found only within the neurosecretory cell groups. Generally they show little sign of secretory activity but differ from the ordinary ganglion cells of the eyestalk in that they possess more cytoplasm and cell boundaries which are easily demonstrated by the techniques used in these studies. The boundaries of the ordinary ganglion cells are extremely difficult to detect with these techniques.

The distribution of neurosecretory cell types is shown in Figure 8. It should be noted that certain cell groups of the crayfish differ in their distribution from that reported in a previous account (Bliss, Durand and Welsh, 1954). The earlier account is essentially correct. However, groups B2 and B3 of the earlier account most likely constitute one group of cells. The group was previously reported to lie lateral to the olfactory lobes. Actually it is located medial to the olfactory lobes on the lateral side of the main mass of fibers of the brain. This distribution of neurosecretory cells brings the neurosecretory system of the crayfish into fairly close agreement with that of *Sesarma* (Enami, 1951).

Secretory activity

No published observations on the normal molting cycle of *O. virilis* are available. However, the following information, although incomplete, shows that there is a single molting time per year for crayfish of the size and sex used in this study. All animals collected on June 28 were soft; the cuticle was parchment-like. Considerable resorption of calcium had occurred from all parts of the exoskeleton and especially from the ischiopodite of the cheliped. Further, all of the animals possessed well developed gastroliths, about 3 mm. in diameter, contained within the gastrolith sac.

Similarly, all animals collected on July 23 were soft; their exoskeletons were

thin and parchment-like, but none possessed any signs of gastroliths. All animals collected on August 14 had hard exoskeletons and showed no signs of an approaching molt.

Since the gastroliths disappear very shortly after molt, the observations indicate that these animals had molted some time between June 28 and July 23. Furthermore, the observations show that the adult male animals used in this study were highly synchronized in their molting period, for none appeared to be approaching a molt on any date after June 28.

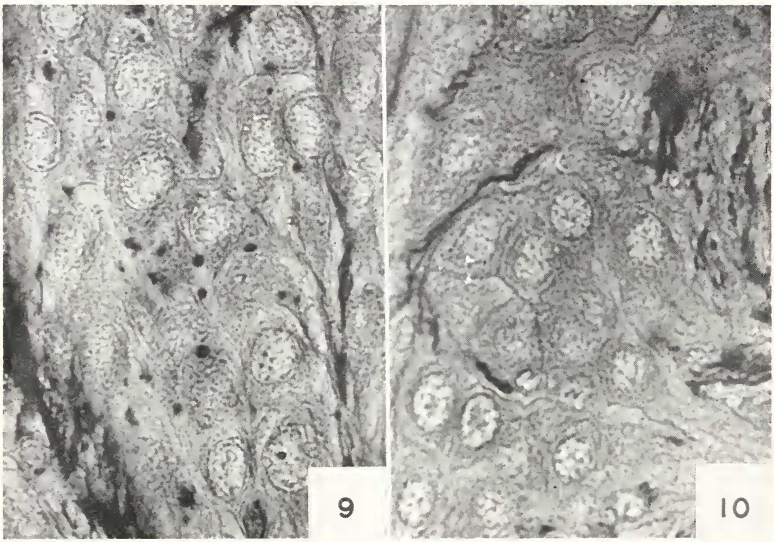


FIGURE 9. Type 2 neurosecretory cells in the x-organ of a crayfish just prior to molt. Note the large number of secretory droplets. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 700 ×.

FIGURE 10. Type 2 neurosecretory cells in the x-organ of a crayfish shortly after molt. Note the lack of secretory material. Bouin's plus 1% calcium chloride; permanganate-aldehyde-fuchsin; 700 ×.

For the greater part of the year, the number of cells that contained secretory material was remarkably constant. However, a striking increase in the number of Type 2 cells that contained droplets of secretory material took place some time before May 6. From Table I it will be observed that more than twice as many Type 2 cells contained secretory droplets on May 6 and June 28 (Fig. 9) than at any other time of sampling (Fig. 10).

Table I shows that the number of Type 1 cells that contained secretory material did not change appreciably throughout the year.

The other neurosecretory cell groups in the eyestalk were examined carefully, but no apparent histological changes occurred in these secretory cells during the

year. The scarcity of secretory material, relative to the amounts present in cell Types 1 and 2, and its occurrence as small granules made it difficult to determine what proportion of cells showed secretory activity. It was concluded, however, that no major histological changes occurred in the other neurosecretory cell groups in the course of this study.

Discussion

Cell types

A few comments should be made regarding a comparison of the neurosecretory cell types of the crayfish with those described for other crustaceans by Enami (1951), Matsumoto (1954), and Carlisle and Passano (1953). Although these authors studied brachyurans and used fixatives other than those used by the present writer, their findings bear similarities with those reported here for the crayfish.

Table I

Counts of Type 1 and Type 2 neurosecretory cells containing secretory material in the brain and eyestalk of Orconectes virilis

Date	Number of animals	Cell type	Mean count and standard error
May 6	2	2	117±1
June 28	4	1	43±1
	4	2	125±8
July 23	4	1	46±2
	4	2	53±5
August 14	3	1	51±2
	3	2	66±4
August 31	4	1	38±2
	4	2	38±4
September 22	3	1	40±1
	3	2	38±6

Of the four neurosecretory cell types described above for the crayfish, it has been shown that the Type 1 and Type 2 cells are practically restricted in their distribution to the x-organ; only a few Type 1 cells are found in other neurosecretory cell groups. A study of Enami's figures reveals that in *Sesarma*, the giant beta neurosecretory cell is the only type found in the x-organ of that animal. Smaller beta neurosecretory cells are present as a small paired group in the supraoesophageal ganglion of *Sesarma*. Enami reports that the cytoplasma of the beta neurosecretory cells is fairly homogeneous and of compact appearance, showing but slight contraction upon fixation. It is apparent from other figures in Enami's paper that the Type 2 cells in the crayfish are similar to the giant beta cells in *Sesarma*.

No Type 2 neurosecretory cells were found in the brain of the crayfish. The crab, *Sesarma*, would appear to differ from the crayfish in that the crab possesses a paired group of beta neurosecretory cells in the supraoesophageal ganglion. In addition, Enami describes no cells in *Sesarma* which are comparable to the Type 1 cells of the crayfish.

The Type 3 cells of the crayfish are comparable to the alpha cells of *Sesarma*

in their distribution and in some of their cytological details. In both animals they are found in all neurosecretory cell groups except the x-organ. Both cell types are rich in cytoplasm and are characterized by the presence of vacuoles which are sharply delimited from the cytoplasm.

The Type 4 neurosecretory cells of the crayfish are similar to the gamma cells of *Sesarma*. They correspond in all features to the gamma cells. Small size, little cytoplasm relative to the size of the nucleus, and scarcity of secretory material are characteristic of these cells in both animals.

Of the four neurosecretory cell types described by Matsumoto (1954) for *Eriocheir japonicus*, he compares only his C cells, located in the ventral ganglion, with Enami's beta cells. However, Enami has shown that no beta neurosecretory cells occur in the ventral ganglion of *Sesarma*. Judging from the figures in their papers and the cell types observed in the crayfish, it appears possible that Matsumoto's C cells might be more comparable to Enami's alpha cells and to the crayfish Type 3 cells.

Carlisle and Passano (1953) found three types of neurosecretory cells in the x-organs of most species of crustaceans they examined. However, the number of cell types later was reduced to two (Carlisle, 1953). These authors showed that in the Natantia, the x-organ is divided into two portions, the pars ganglionaris which is located on the medulla terminalis and the pars distalis which is located elsewhere in the eyestalk. The Brachyura and the crayfish, in contrast to the Natantia, possess an undivided x-organ. Also, Carlisle and Passano found one neurosecretory cell type to be located in the pars ganglionaris x-organi and the other in the pars distalis x-organi. The cells of the pars ganglionaris x-organi are comparable to the giant beta neurosecretory cells of *Sesarma*, and Carlisle and Passano referred to them as the *x-organ neurosecretory cells*. It is evident that, since the Type 2 neurosecretory cells of the crayfish are comparable to the giant beta neurosecretory cells of *Sesarma*, they are also similar to the *x-organ neurosecretory cells* described in the Natantia by Carlisle and Passano.

There is a close parallelism in the arrangement of neurosecretory cell groups of the crayfish and the land crab, *Gecarcinus* (Bliss, Durand and Welsh, 1954). Furthermore, a comparison of Figure 8 of the present paper with Figure 11 in Enami's paper (1951) has already revealed that there is a remarkable similarity in the distribution of neurosecretory cell types in the crayfish and *Sesarma*. The parallelism in the distribution of neurosecretory cell types in the crayfish and *Sesarma* is particularly interesting when the physiological role of these cells is considered. This is discussed in the next section.

Secretory activity

When considering the increase in secretory activity that was observed in one type of neurosecretory cell in May, it should be remembered that the animals used in May had been kept in the laboratory for three to four months. There is evidence that crustaceans kept in the laboratory for long periods of time are different from those freshly collected. The molt-promoting effects of constant darkness on *Gecarcinus* (Bliss, 1954) are slowed down or delayed when freshly collected crabs are used. Animals long maintained in the laboratory respond quickly (Bliss, personal communication). *O. virilis*, kept in the laboratory, have been observed to

molt in fairly large numbers in May and the first part of June. Although no observations were made on the molting of *O. virilis* in the field in May and in early June, it seems reasonable to assume that the laboratory stock animals molt at an earlier date than animals in the field because of the higher temperatures and more regular food supply that probably exist under laboratory conditions. Therefore, data from the February and May animals used in this study may not be strictly comparable to data from crayfish that were freshly collected.

The results included in Table I raise a question concerning the physiological significance of the increased content of stainable material in the Type 2 neurosecretory cells of the x-organ just prior to molt. The idea that the sinus gland is a storage-release center for neurosecretory products (Bliss, 1951, 1953; Bliss, Durand and Welsh, 1954; Bliss and Welsh, 1952; Passano, 1951a, 1951b, 1952, 1953) implies that there is a mechanism whereby the rate of release of the substances can be controlled. Indeed, the well known reactions of certain crustaceans to background color are evidence that the release of certain neurosecretory products, *e.g.*, chromatophorotropins, is precisely regulated. Since a molt-inhibiting substance is produced in the x-organ and passed to the sinus gland for release into the blood stream, it is necessary to assume that at some time before the animal molts there is a decreased synthesis of this substance in the cells of the x-organ, a decreased release from the sinus gland, or both. It is assumed here that the release of the molt-inhibiting substance is decreased before molt.

It is known that the pars ganglionaris x-organi of the Natantia produces a molt-inhibiting hormone (Carlisle, 1954) and is comparable to a portion of the x-organ in the crayfish (this paper). Since the crayfish x-organ probably produces a molt-inhibiting hormone and since the only neurosecretory cells present in the pars ganglionaris x-organi of the Natantia are comparable to the Type 2 neurosecretory cells of the crayfish, it is conceivable that this neurosecretory cell type is the source of the molt-inhibiting hormone. If this is so, then the accumulation of stainable material found in the Type 2 neurosecretory cells just before molt can be considered evidence of more (1) precursor of the molt-inhibitor substance, (2) carrier substance, or (3) active material.

It is evident from the cell counts of the May animals that an assumed reduction in the rate of release must occur over a rather long period before molt. In adult crayfish large amounts of secretory material are present in these cells early in May, and signs of this increase are found in February in laboratory crayfish. Preliminary studies show that in immature crayfish possessing an intermolt period of approximately thirty-five days, increased amounts of secretory material are present in Type 2 neurosecretory cells of the x-organ at least five days before molt.

Fewer Type 2 neurosecretory cells contain secretory material after molt. This could result, if, after molt, there is a sudden release of stored material from the sinus gland and a rapid transfer of material from the cell bodies to the sinus gland for further release. Pyle (1943) found pronounced changes after molt in both the amount and staining qualities of the sinus gland material. He fixed eyestalks from *O. virilis* a few hours before molt and after the animals had completed molt. He found that there was a sharp reduction in the number of secretory granules present in the sinus glands after molt. Since the secretory masses he refers to in his photographs are identical in appearance with similar masses observed by the present

author in neurosecretory fiber endings in the sinus glands, it is possible that practically all of the material in a given axon ending is released after molt. In the crayfish this release takes place in a period of not more than a few hours (Pyle, 1943).

The accumulation of stainable material in the Type 2 neurosecretory cells of the x-organ prior to molt has been explained on the basis of a hypothetical witholding of molt-inhibiting hormone by the sinus gland and a continued synthesis of hormone or its precursor in Type 2 neurosecretory cell bodies of the x-organ. The secretory

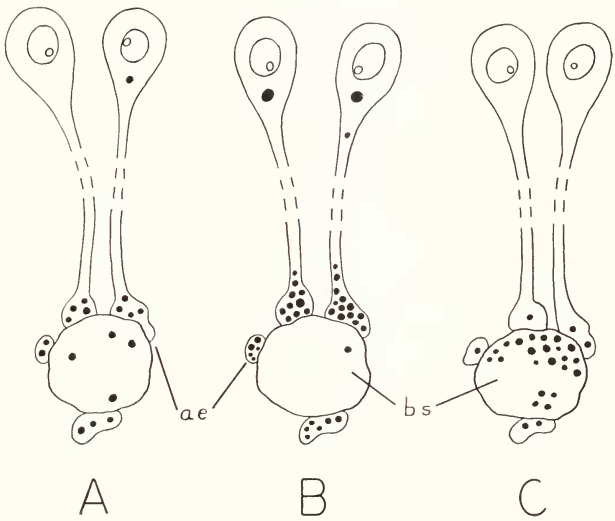


FIGURE 11. Hypothetical scheme for the secretory activity of Type 2 neurosecretory cells. A. During the intermolt period, a slow release of secretory material into the blood and synthesis of the material in Type 2 neurosecretory cell bodies continues. B. Shortly before molt, release of neurosecretory material into the blood is decreased; synthesis of the material in the Type 2 neurosecretory cell bodies continues. Material thus accumulates in the axon endings and in the cell bodies. C. Immediately after molt, a sudden release of secretory material into the blood occurs; cell body secretory material is transferred quickly to the axon endings for release. ae, axon ending; bs, blood sinus.

activity of the Type 2 neurosecretory cells is summarized in Figure 11. This is in complete agreement with the existing hypothesis on the control of molt in crustaceans (Bliss, 1953; Bliss, Durand and Welsh, 1954; Bliss and Welsh, 1952; Passano, 1953). It is interesting that the only neurosecretory cells of the eyestalk that show histological changes correlated with molt are restricted to the x-organ, the only cell group so far proved to be effective in the prevention of molt (Passano, 1953). As to the functions of the other neurosecretory cell types in the crayfish, no information was obtained in this study.

Summary

1. There are four cytologically distinct types of neurosecretory cells in the eyestalk and brain of *Orconectes virilis*. Two of these neurosecretory cell types are restricted in their distribution to the x-organ. The other two cell types occur in all neurosecretory cell groups in the eyestalk and brain except the x-organ.

2. The distribution of neurosecretory cell types has been compared with that described by Enami (1951) for *Sesarma*.

3. The Type 2 neurosecretory cells are the only neurosecretory cells that undergo histologically demonstrable changes in secretory activity in relation to the molting cycle. It is suggested, therefore, that the Type 2 neurosecretory cells are the source of the molt-inhibiting hormone.

4. Arguments are presented in favor of the view that at some time before molt a decrease occurs in the rate of release of molt-inhibiting hormone from the axon endings of the Type 2 neurosecretory cells. This decrease seems to be correlated with a concurrent accumulation of stainable material observed in Type 2 neurosecretory cell bodies.

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